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THE EXTRACTION OF LUMBRICIDAE FROM SOIL WITH SPECIAL REFERENCE TO THE HAND-SORTING METHOD

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A brief review is given of chemical methods and the use of an electric current for recovering earthworms from soil. Tests of the efficiency of hand-sorting with grassland samples indicate that underestimates of the numbers of the smaller specimens and those having a dark coloration are likely to occur. The presence of inactive worms and large amounts of semi-decayed organic material may also affect the efficiency of the method. It is essential to have a means of checking the accuracy of the operator; the introduction of small, living worms injected with methylene blue is suitable for this purpose.

THREE methods of extracting earthworms are currently used: chemical methods, the use of an electric current and hand-sorting. Some brief comments are made on these methods and details are given of an investigation on the efficiency of hand-sorting.

EXTRACTION METHODS

Chemical Methods

The application of liquid vermifuges to the soil has been described by EVANS and GUILD (1947) who used potassium permanganate solution and by JEFFERSON (1955–8) who used Mowrah meal*. The use of chemical expellents has been criticized (SATCHELL 1955; SVENDSEN, 1955) on the grounds that vermifuges extract only part of the population in the sample area, and are to some extent selective in that they expel species with wide burrows and a vigorous response to stimulation more effectively than other species. Nevertheless the method is convenient and rapid and has provided valuable results in field surveys where precise population estimates were not required.

Electrical Method

A sampling method in which the earthworms are expelled electrically has been described (SATCHELL, 1955) for use, for example, in land devoted to fertilizer experiments where the application of chemical expellents or the removal of soil samples would disturb the experiment. The method as originally described required a mains-electricity supply but it has recently been used successfully with a generator (2 kVA, a.c.) mounted on the back of a Land-Rover and connected by a pulley belt to the power take-off of the vehicle (Barley, *in lit.*). The main defect of the electrical method is the difficulty of defining the exact limits of the volume of soil which has been effectively treated.

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* Mowrah meal is the material remaining after the expression of oil from the ground seeds of the Bassia tree (*Bassia longifolia* L.) and contains a saponin-like substance—Ed.

Hand-Sorting

A more widely used method is that of removing samples of soil from the field and sorting out the worms by hand. Svendsen (1955), in a comparison of hand-sorting and the potassium permanganate method, obtained almost twice as many worms by the former method. Furthermore, few of the population estimates obtained in Britain by the use of potassium permanganate have exceeded 0·5 million per acre (123/m^2), whereas with hand-sorting, Ford (1935), Low (1955), Reynoldson (1955), Reynoldson, O'Connor and Kelly (1955) and Svendsen (1955) obtained estimates of 1·5–3 million per acre (369–738/m^2). There appears, however, to be no published work assessing the efficiency of hand-sorting although it has been used as a basis for judging the effectiveness of other methods of extracting earthworms from the soil. We have therefore tested the method using prepared samples containing known numbers of earthworms. A further test was carried out on natural samples obtained from the field.

HAND-SORTING PREPARED SAMPLES

Known numbers of worms of various species differing in size and pigmentation were placed in samples of worm-free soil which were later sorted by operators who were unaware of the numbers of worms introduced. Square blocks of soil (10-in. sides and 2 in. deep; 25·4 × 5 cm, *ca.* 650 cm^2) were cut from the surface of a rough matted grassland and frozen at about -16°C to kill all the earthworms. Fertile pasture soil was also freed of worms by fumigation with carbon tetrachloride. One block and sufficient soil to form a cube (10-in. sides; 25·4 cm) was then enclosed in each of 30 bags of fine-mesh nylon cloth. These were placed in a shallow pit so that the tops of the turves were level with the ground surface, and left for a week to reach the moisture level of the surrounding soil. Thirty-three earthworms (equivalent to *ca.* 2 million/acre; 492/m^2) were then placed in each bag (*Table 56*). The following day a few damaged worms which had failed to burrow into the turf were replaced.

The samples were hand-sorted in the laboratory by two operators at intervals of 10–36 days after the addition of the earthworms. The first bag sorted by each was treated as a practice sample and discarded so that the results (*Table 56*) are based on 28 samples. Some loss of worms from mortality during the course of the experiment was expected. Samples from which worms appeared to be missing were therefore re-examined by a third operator who recovered 70 per cent of the missing worms (*Table 56*).

These results provide no evidence of any serious loss in the recovery by hand-sorting of large and medium sized species but the recovery of the smaller worms by this method appears to be unreliable. Factors such as small size, dark coloration, immobility and presence in the turf rather than in the soil, appeared to militate against their detection. The loss of immature specimens of *Allolobophora chlorotica* (Sav.) which were pigmented and inactive was more than twice that of the immature *Eisenia rosea* (Sav.), which were unpigmented and moderately active. Both species occurred mainly in the soil. *Lumbricus castaneus* (Sav.), the recovery of which was even lower than that of the immature *A. chlorotica*, is an active species but is deeply pigmented and occurred mainly in the turf. Of the 15 specimens which were missed in the first sorting but were found subsequently, one was missed in each of 10 samples, two were missed in one, and, in another sample, three of the 4 specimens present were overlooked.

Hand-sorting is clearly unsuitable for studies involving the smaller species or very immature worms, but the evidence of this experiment suggests that estimates of total numbers or biomass are not generally likely to be seriously inaccurate as a result of losses due to sorting. Of the 924 earthworms introduced, 93 per cent (99 per cent by weight) were recovered in the first sorting and of the missing 7 per cent, about one-third were not found in the second sorting, and these included some which had died during the experiment.

Table 56. Number of earthworms recovered by hand-sorting from prepared grassland samples containing known numbers of worms
(total number from 28 samples; sample size 10 × 10 in. (25 cm) and 10 in. deep)

Species	Remarks	Mean weight per individual* (g)	Number introduced per sample	Number found first sorting (per cent)	Number found second sorting (per cent)
Pigmented species					
Lumbricus terrestris L.		2·27	1	100	0
Allolobophora longa Ude		1·69	4	100	0
,, *chlorotica* (Sav.)	Green form	0·21	6	95	2
,, ,,	Green form Immature	0·10	2	80	14
Lumbricus castaneus (Sav.)		0·07	4	74	20
Unpigmented species					
Octolasium cyaneum (Sav.)		1·68	1	96	0
Allolobophora caliginosa (Sav.)		0·42	5	100	0
,, *chlorotica* (Sav.)	Pink form	0·25	6	98	2
Eisenia rosea (Sav.)	Immature	0·07	4	91	6

* Average of 100 individuals weighed together.

HAND-SORTING NATURAL GRASSLAND SAMPLES

Since the method of hand-sorting by one operator and re-sorting by a second had succeeded in recovering 98 per cent of the worms from the prepared samples, the method was applied to natural samples taken from the field. Ten sampling units (10-in. sides and 10 in. deep; 25 cm) were dug out of a pasture field from well-dunged areas where the earthworm population appeared to be high. The units were divided into two groups of five, each being sorted by a separate operator and checked by a third. The total number of worms recovered, and the proportion found in the re-examination by an independent sorter, are given in *Table 57*, where the species found are grouped in descending order of their approximate mean weights.

The results agree with those from the prepared samples in that immature specimens of *A. chlorotica* and *L. castaneus*, the latter in this case tabulated with *Dendrobaena mammalis* (Sav.) which it closely resembles in size and pigmentation, were apparently least efficiently recovered by the initial hand-sorting. The proportion of the total catch recovered in the second hand-sorting was again small, being 2·9 per cent of the total number and 1·0 per cent by weight.

Table 57. Number of earthworms obtained by hand-sorting from natural grassland (total number from 10 sampling units; unit size 10 × 10 in. (25 cm) and 10 in. deep)

Species groups in order of approximate mean weights*	Maturity	Total number found	Number found second sorting (per cent)
Allolobophora caliginosa (Sav.) ,, *limicola* Mich. ,, *longa* Ude *Eisenia rosea* (Sav.) *Lumbricus rubellus* Hoff. ,, *terrestris* L. *Octolasium lacteum* Örley	Adult	92	1·1
Allolobophora longa Ude *Lumbricus rubellus* Hoff. ,, *terrestris* L.	Immature	178	1·1
Allolobophora chlorotica (Sav.)	Adult	77	1·3
Dendrobaena mammalis (Sav.) *Lumbricus castaneus* (Sav.)	Adult	146	2·7
Allolobophora caliginosa (Sav.) ,, *limicola* Mich. *Eisenia rosea* (Sav.) *Octolasium lacteum* Örley	Immature	59	1·7
Dendrobaena mammalis (Sav.) *Lumbricus castaneus* (Sav.)	Immature	81	4·9
Allolobophora chlorotica (Sav.)	Immature	65	10·8
Total		698	2·9
Total weight (g)		260	1·0

* See *Table 56* for mean weights of individual species.

For certain types of study, hand-sorting appears to show promise for field sites where the removal of adequate soil samples is possible. These will not include the earthworms occupying the subsoil at a depth which it is impracticable to sample but the possibility of estimating their numbers by using an expellent after removing the topsoil might be considered. Our experience of hand-sorting suggests, however, that it is highly unreliable if used without adequate checks. In these experiments the two sorters, although they were unaware of the number of worms to be expected in the samples, knew that their samples would be re-sorted, and this undoubtedly contributed to their efficiency (*Table 58*). Despite this, operator B completed his examination of one prepared sample—subsequently discarded—in 45 minutes, i.e. three-fifths of his average time (*Table 58*), and in doing so missed 25 per cent of the worms eventually recovered.

Table 58. Comparison of the efficiency of two operators in hand-sorting earthworms from grassland samples
(for sampling details see *Tables 56* and *57*)

Operator	Average time per sample (min)	Worms recovered in second sorting	
		Proportion of total number (per cent)	Weight (g)
Prepared samples			
A	143	3·3	0·6
B	80	6·7	1·4
Natural samples			
A	259	2·6	0·6
B	134	3·2	2·1

Sorting soil by hand is extremely tedious and exacting work and a method of checking the reliability of each sorting is essential. A second sorting of the sample, when it contains few worms, is more time consuming and even more tedious than the initial sorting and can hardly be carried out as a routine measure. The introduction of marked living worms to each sample some hours before sorting is suggested as an alternative. The number introduced should be unknown to the sorter since efficiency may fall once all the marked worms have been recovered. Sampling errors due to inefficient sorting can then be kept at a low level by rejecting or re-sorting samples in which a fixed minimum recovery of marked worms has not been achieved. Small specimens of *A. chlorotica*, injected with methylene blue, are satisfactory as marker worms.

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